Tobacco Rattle Virus Silencing Vector

RNA-1: LSB-1

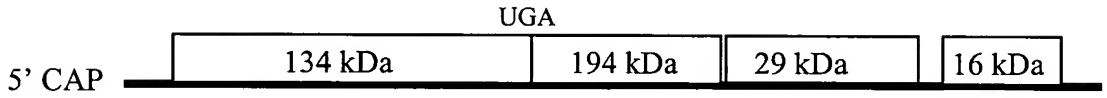

FIG. 1A

RNA-2: PpK20

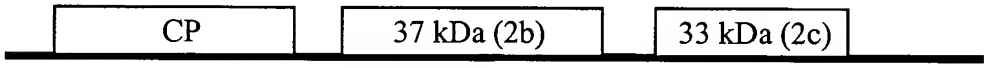

FIG. 1B

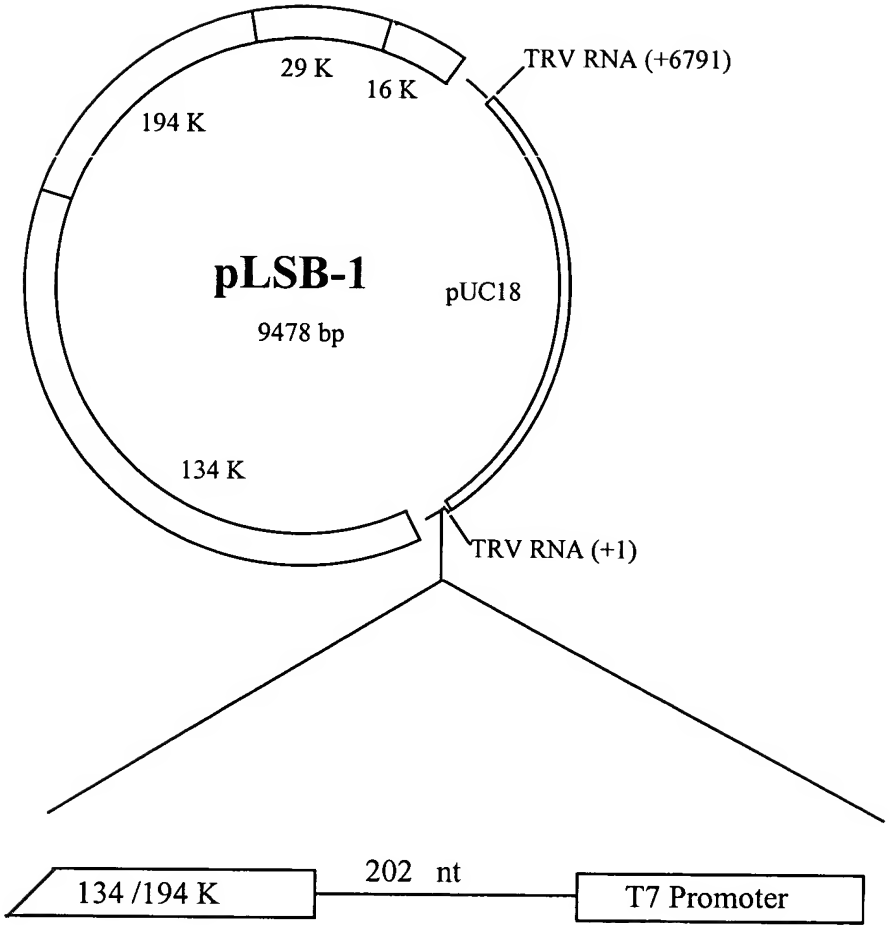
29 K
16 K
TRV RNA (+6791)
194 K
pLSB-1
pUC18
9478 bp
134 K
TRV RNA (+1)
134 /194 K
202 nt
T7 Promoter

FIG. 2

ATAAAACATTTCAATCCTTTGAACGCGGTAGAACGTGCTAATTGGATTTTGGTGAGAA
CGCGGTAGAACGTACTTATCACCTACAGTTTTATTTTGTTTTTCTTTTTGGTTTAATCTA
TCCAGCTTAGTACCGAGTGGGGGAAAGTGACTGGTGTGCCTAAAACCTTTTCTTTGAT
ACTTTGTAAAAATACATACAGATACAATGGCGAACGGTAACTTCAAGTTGTCTCAATT
GCTCAATGTGGACGAGATGTCTGCTGAGCAGAGGAGTCATTTCTTTGACTTGATGCTG
ACTAAACCTGATTGTGAGATCGGGCAAATGATGCAAAGAGTTGTTGTTGATAAAGTCG
ATGACATGATTAGAGAAAGAAAGACTAAAGATCCAGTGATTGTTCATGAAGTTCTTTC
TCAGAAGGAACAGAACAAGTTGATGGAAATTTATCCTGAATTCAATATCGTGTTTAAA
GACGACAAAAACATGGTTCATGGGTTTGCGGCTGCTGAGCGAAAACTACAAGCTTTAT
TGCTTTTAGATAGAGTTCCTGCTCTGCAAGAGGTGGATGACATCGGTGGTCAATGGTC
GTTTTGGGTAACTAGAGGTGAGAAAAGGATTCATTCCTGTTGTCCAAATCTAGATATT
CGGGATGATCAGAGAGAAATTTCTCGACAGATATTTCTTACTGCTATTGGTGATCAAG
CTAGAAGTGGTAAGAGACAGATGTCGGAGAATGAGCTGTGGATGTATGACCAATTTC
GTGAAAATATTGCTGCGCCTAACGCGGTTAGGTGCAATAATACATATCAGGGTTGTAC
ATGTAGGGGTTTTTCTGATGGTAAGAAGAAAGGCGCGCAGTATGCGATAGCTCTTCAC
AGCCTGTATGACTTCAAGTTGAAAGACTTGATGGCTACTATGGTTGAGAAGAAAACTA
AAGTGGTTCATGCTGCTATGCTTTTTGCTCCTGAAAGTATGTTAGTGGACGAAGGTCC
ATTACCTTCTGTTGACGGTTACTACATGAAGAAGAACGGGAAGATCTATTTCGGTTTT
GAGAAAGATCCTTCCTTTTCTTACATTCATGACTGGGAAGAGTACAAGAAGTATCTAC
TGGGGAAGCCAGTGAGTTACCAAGGGGATGTGTTCTACTTCGAACCGTGGCAGGTGA
GAGGAGACACAATGCTTTTTTCGATCTACAGGATAGCTGGAGTTCCGAGGAGGTCTCT
ATCATCGCAAGAGTACTACCGAAGAATATATATCAGTAGATGGGAAAACATGGTTGT
TGTCCCAATTTTCGATCTGGTCGAATCAACGCGAGAGTTGGTCAAGAAAGACCTGTTT
GTAGAGAAACAATTCATGGACAAGTGTTTGGATTACATAGCTAGGTTATCTGACCAGC
AGCTGACCATAAGCAATGTTAAATCATACTTGAGTTCAAATAATTGGGTCTTATTCAT
AAACGGGGCGGCCGTGAAGAACAAGCAAAGTGTAGATTCTCGAGATTTACAGTTGTT
GGCTCAAACTTTGCTAGTGAAGGAACAAGTGGCGAGACCTGTCATGAGGGAGTTGCG
TGAAGCAATTCTGACTGAGACGAAACCTATCACGTCATTGACTGATGTGCTGGGTTTA
ATATCAAGAAAACTGTGGAAGCAGTTTGCTAACAAGATCGCAGTCGGCGGATTCGTT
GGCATGGTTGGTACTCTAATTGGATTCTATCCAAAGAAGGTACTAACCTGGGCGAAGG
ACACACCAAATGGTCCAGAACTATGTTACGAGAACTCGCACAAAACCAAGGTGATAG
TATTTCTGAGTGTTGTGTATGCCATTGGAGGAATCACGCTTATGCGTCGAGACATCCG
AGATGGACTGGTGAAAAAACTATGTGATATGTTTGATATCAAACGGGGGGCCCATGT
CTTAGACGTTGAGAATCCGTGCCGCTATTATGAAATCAACGATTTCTTTAGCAGTCTGT

FIG. 3A

ATTCGGCATCTGAGTCCGGTGAGACCGTTTTACCAGATTTATCCGAGGTAAAAGCCAA
GTCTGATAAGCTATTGCAGCAGAAGAAAGAAATCGCTGACGAGTTTCTAAGTGCAAA
ATTCTCTAACTATTCTGGCAGTTCGGTGAGAACTTCTCCACCATCGGTGGTCGGTTCAT
CTCGAAGCGGACTGGGTCTGTTGTTGGAAGACAGTAACGTGCTGACCCAAGCTAGAG
TTGGAGTTTCAAGAAAGGTAGACGATGAGGAGATCATGGAGCAGTTTCTGAGTGGTC
TTATTGACACTGAAGCAGAAATTGACGAGGTTGTTTCAGCCTTTTCAGCTGAATGTGA
AAGAGGGGAAACAAGCGGTACAAAGGTGTTGTGTAAACCTTTAACGCCACCAGGATT
TGAGAACGTGTTGCCAGCTGTCAAACCTTTGGTCAGCAAAGGAAAAACGGTCAAACG
TGTCGATTACTTCCAAGTGATGGGAGGTGAGAGATTACCAAAAAGGCCGGTTGTCAGT
GGAGACGATTCTGTGGACGCTAGAAGAGAGTTTCTGTACTACTTAGATGCGGAGAGA
GTCGCTCAAAATGATGAAATTATGTCTCTGTATCGTGACTATTCGAGAGGAGTTATTC
GAACTGGAGGTCAGAATTACCCGCACGGACTGGGAGTGTGGGATGTGGAGATGAAGA
ACTGGTGCATACGTCCAGTGGTCACTGAACATGCTTATGTGTTCCAACCAGACAAACG
TATGGATGATTGGTCGGGATACTTAGAAGTGGCTGTTTGGGAACGAGGTATGTTGGTC
AACGACTTCGCGGTCGAAAGGATGAGTGATTATGTCATAGTTTGCGATCAGACGTATC
TTTGCAATAACAGGTTGATCTTGGACAATTTAAGTGCCCTGGATCTAGGACCAGTTAA
CTGTTCTTTTGAATTAGTTGACGGTGTACCTGGTTGTGGTAAGTCGACAATGATTGTCA
ACTCAGCTAATCCTTGTGTCGATGTGGTTCTCTCTACTGGGAGAGCAGCAACCGACGA
CTTGATCGAGAGATTCGCGAGCAAAGGTTTTCCATGCAAATTGAAAAGGAGAGTGAA
GACGGTTGATTCTTTTTTGATGCATTGTGTCGATGGTTCTTTAACCGGAGACGTGTTGC
ATTTCGACGAAGCTCTCATGGCCCATGCTGGTATGGTGTACTTTTGCGCTCAGATAGCT
GGTGCTAAACGATGTATCTGTCAAGGAGATCAGAATCAAATTTCTTTCAAGCCTAGGG
TATCTCAAGTTGATTTGAGGTTTTCTAGTCTGGTCGGAAAGTTTGACATTGTTACAGAA
AAAAGAGAAACTTACAGAAGTCCAGCAGATGTGGCTGCCGTATTGAACAAGTACTAT
ACTGGAGATGTCAGAACACATAACGCGACTGCTAATTCGATGACGGTGAGGAAGATT
GTGTCTAAAGAACAGGTTTCTTTGAAGCCCGGTGCTCAGTACATAACTTTCCTTCAGTC
TGAGAAGAAGGAGTTGGTAAATTTGTTGGCATTGAGGAAAGTGGCAGCTAAAGTGAG
TACAGTACACGAGTCGCAAGGAGAGACATTCAAAGATGTAGTCCTAGTCAGGACGAA
ACCTACGGATGACTCAATCGCTAGAGGTCGGGAGTACTTAATCGTGGCGTTGTCGCGT
CACACACAATCACTTGTGTATGAAACTGTGAAAGAGGACGATGTAAGCAAAGAGATC
AGGGAAAGTGCCGCGCTTACGAAGGCGGCTTTGGCAAGATTTTTTGTTACTGAGACCG
TCTTATGACGGTTTCGGTCTAGGTTTGATGTCTTTAGACATCATGAAGGGCCTTGCGCC
GTTCCAGATTCAGGTACGATTACGGACTTGGAGATGTGGTACGACGCTTTGTTTCCGG
GAAATTCGTTAAGAGACTCAAGCCTAGACGGGTATTTGGTGGCAACGACTGATTGCA

FIG. 3B

ATTTGCGATTAGACAATGTTACGATCAAAAGTGGAAACTGGAAAGACAAGTTTGCTG
AAAAAGAAACGTTTCTGAAACCGGTTATTCGTACTGCTATGCCTGACAAAAGGAAGA
CTACTCAGTTGGAGAGTTTGTTAGCATTGCAGAAAAGGAACCAAGCGGCACCCGATCT
ACAAGAAAATGTGCACGCGACAGTTCTAATCGAAGAGACGATGAAGAAGCTGAAATC
TGTTGTCTACGATGTGGGAAAAATTCGGGCTGATCCTATTGTCAATAGAGCTCAAATG
GAGAGATGGTGGAGAAATCAAAGCACAGCGGTACAGGCTAAGGTAGTAGCAGATGT
GAGAGAGTTACATGAAATAGACTATTCGTCTTACATGTATATGATCAAATCTGACGTG
AAACCTAAGACTGATTTAACACCGCAATTTGAATACTCAGCTCTACAGACTGTTGTGT
ATCACGAGAAGTTGATCAACTCGTTGTTCGGTCCAATTTTCAAAGAAATTAATGAACG
CAAGTTGGATGCTATGCAACCACATTTTGTGTTCAACACGAGAATGACATCGAGTGAT
TTAAACGATCGAGTGAAGTTCTTAAATACGGAAGCGGCTTACGACTTTGTTGAGATAG
ACATGTCTAAATTCGACAAGTCGGCAAATCGCTTCCATTTACAACTGCAGCTGGAGAT
TTACAGGTTATTTGGGCTGGATGAGTGGGCGGCCTTCCTTTGGGAGGTGTCGCACACT
CAAACTACTGTGAGAGATATTCAAAATGGTATGATGGCGCATATTTGGTACCAACAAA
AGAGTGGAGATGCTGATACTTATAATGCAAATTCAGATAGAACACTGTGTGCGCTCTT
GTCTGAATTACCATTGGAGAAAGCAGTCATGGTTACATATGGAGGAGATGACTCACTG
ATTGCGTTTCCTAGAGGAACGCAGTTTGTTGATCCGTGTCCAAAGTTGGCTACTAAGT
GGAATTTCGAGTGCAAGATTTTTAAGTACGATGTCCCAATGTTTTGTGGGAAGTTCTT
GCTTAAGACGTCATCGTGTTACGAGTTCGTGCCAGATCCGGTAAAAGTTCTGACGAAG
TTGGGGAAAAAGAGTATAAAGGATGTGCAACATTTGGCCGAGATCTACATCTCGCTG
AATGATTCCAATAGAGCTCTTGGGAACTACATGGTGGTATCCAAACTGTCCGAGTCTG
TTTCAGACCGGTATTTGTACAAAGGTGATTCTGTTCATGCGCTTTGTGCGCTATGGAAG
CATATTAAGAGTTTTACAGCTCTGTGTACATTATTCCGAGACGAAAACGATAAGGAAT
TGAACCCGGCTAAGGTTGATTGGAAGAAGGCACAGAGAGCTGTGTCAAACTTTTACG
ACTGGTAATATGGAAGACAAGTCATTGGTCACCTTGAAGAAGAAGACTTTCGAAGTCT
CAAAATTCTCAAATCTAGGGGCCATTGAATTGTTTGTGGACGGTAGGAGGAAGAGAC
CGAAGTATTTTCACAGAAGAAGAGAAACTGTCCTAAATCATGTTGGTGGGAAGAAGA
GTGAACACAAGTTAGACGTTTTTGACCAAAGGGATTACAAAATGATTAAATCTTACGC
GTTTCTAAAGATAGTAGGTGTACAACTAGTTGTAACATCACATCTACCTGCAGATACG
CCTGGGTTCATTCAAATCGATCTGTTGGATTCGAGACTTACTGAGAAAAGAAAGAGAG
GAAAGACTATTCAGAGATTCAAAGCTCGAGCTTGCGATAACTGTTCAGTTGCGCAGTA
CAAGGTTGAATACAGTATTTCCACACAGGAGAACGTACTTGATGTCTGGAAGGTGGGT
TGTATTTCTGAGGGCGTTCCGGTCTGTGACGGTACATACCCTTTCAGTATCGAAGTGTC
GCTAATATGGGTTGCTACTGATTCGACTAGGCGCCTCAATGTGGAAGAACTGAACAGT

FIG. 3C

TCGGATTACATTGAAGGCGATTTTACCGATCAAGAGGTTTTCGGTGAGTTCATGTCTTT
GAAACAAGTGGAGATGAAGACGATTGAGGCGAAGTACGATGGTCCTTACAGACCAGC
TACTACTAGACCTAAGTCATTATTGTCAAGTGAAGATGTTAAGAGAGCGTCTAATAAG
AAAAACTCGTCTTAATGCATAAAGAAATTTATTGTCAATATGACGTGTGTACTCAAGG
GTTGTGTGAATGAAGTCACTGTTCTTGGTCACGAGACGTGTAGTATCGGTCATGCTAA
CAAATTGCGAAAGCAAGTTGCTGACATGGTTGGTGTCACACGTAGGTGTGCGGAAAA
TAATTGTGGATGGTTTGTCTGTGTTGTTATCAATGATTTTACTTTTGATGTGTATAATTG
TTGTGGCCGTAGTCACCTTGAAAAGTGTCGTAAACGTGTTGAAACAAGAAATCGAGA
AATTTGGAAACAAATTCGACGAAATCAAGCTGAAAACATGTCTGCGACAGCTAAAAA
GTCTCATAATTCGAAGACCTCTAAGAAGAAATTCAAAGAGGACAGAGAATTTGGGAC
ACCAAAAAAGATTTTTAAGAGATGATGTTCCTTTCGGGATTGATCGTTTGTTTGCTTTTT
GATTTTATTTTATATTGTTATCTGTTTCTGTGTATAGACTGTTTGAGATTGGCGCTTGGC
CGACTCATTGTCTTACCATAGGGGAACGGACTTTGTTTGTGTTGTTATTTTATTTGTAT
TTTATTAAAATTCTCAATGATCTGAAAAGGCCTCGAGGCTAAGAGATTATTGGGGGGT
GAGTAAGTACTTTTAAAGTGATGATGGTTACAAAGGCAAAAGGGGTAAAACCCCTCG
CCTACGTAAGCGTTATTACGCCC-3' (SEQ ID NO: X).

FIG. 3D

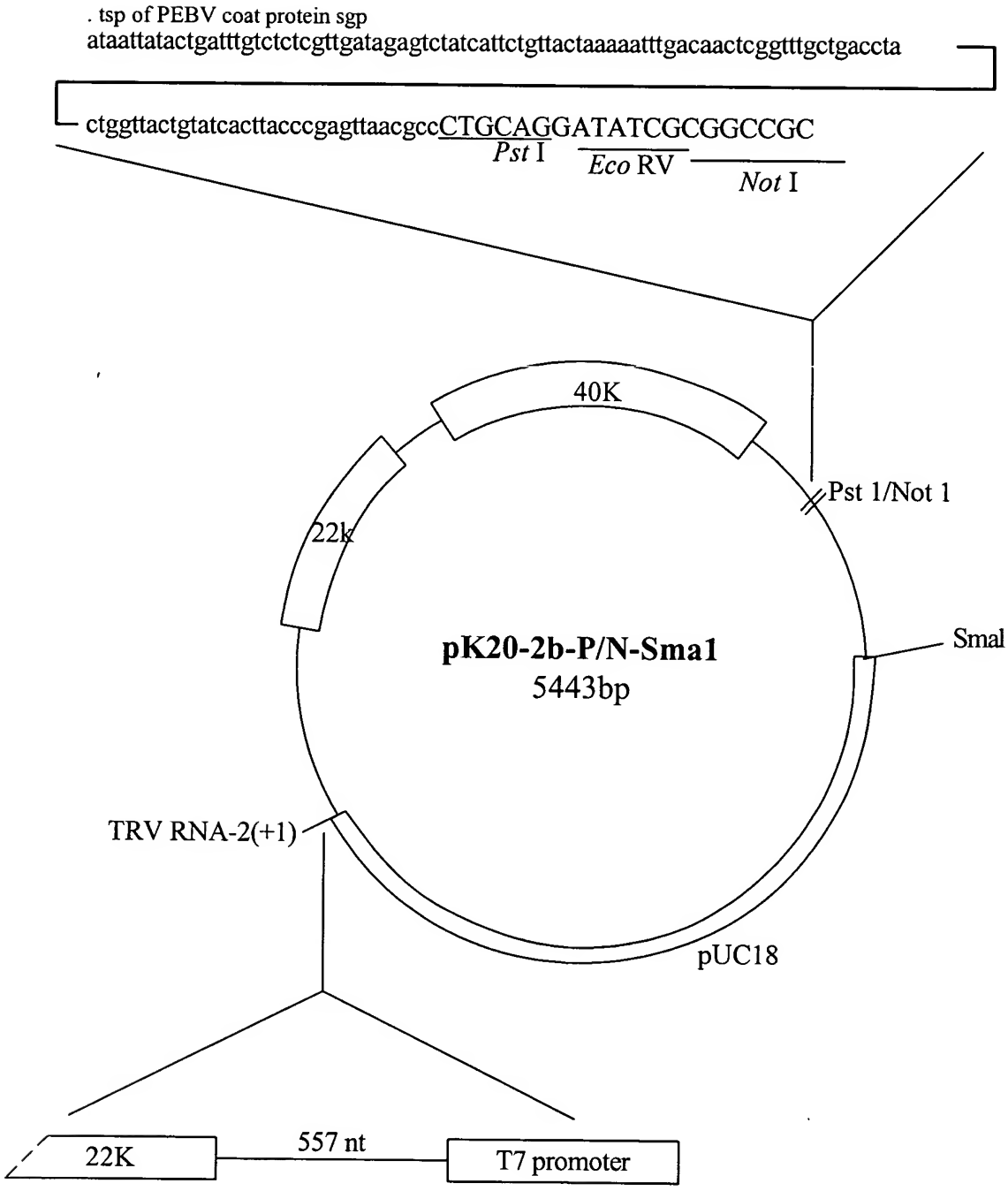
. tsp of PEBV coat protein sgp
ataattatactgatttgtctctcgttgatagagtctatcattctgttactaaaaatttgacaactcggtttgctgaccta
ctggttactgtatcacttacccgagttaacgccCTGCAGGATATCGCGGCCGC
Pst I
Eco RV
Not I
40K
22k
Pst 1/Not 1
SmaI
pK20-2b-P/N-Sma1
5443bp
TRV RNA-2(+1)
pUC18
22K
557 nt
T7 promoter

FIG. 4

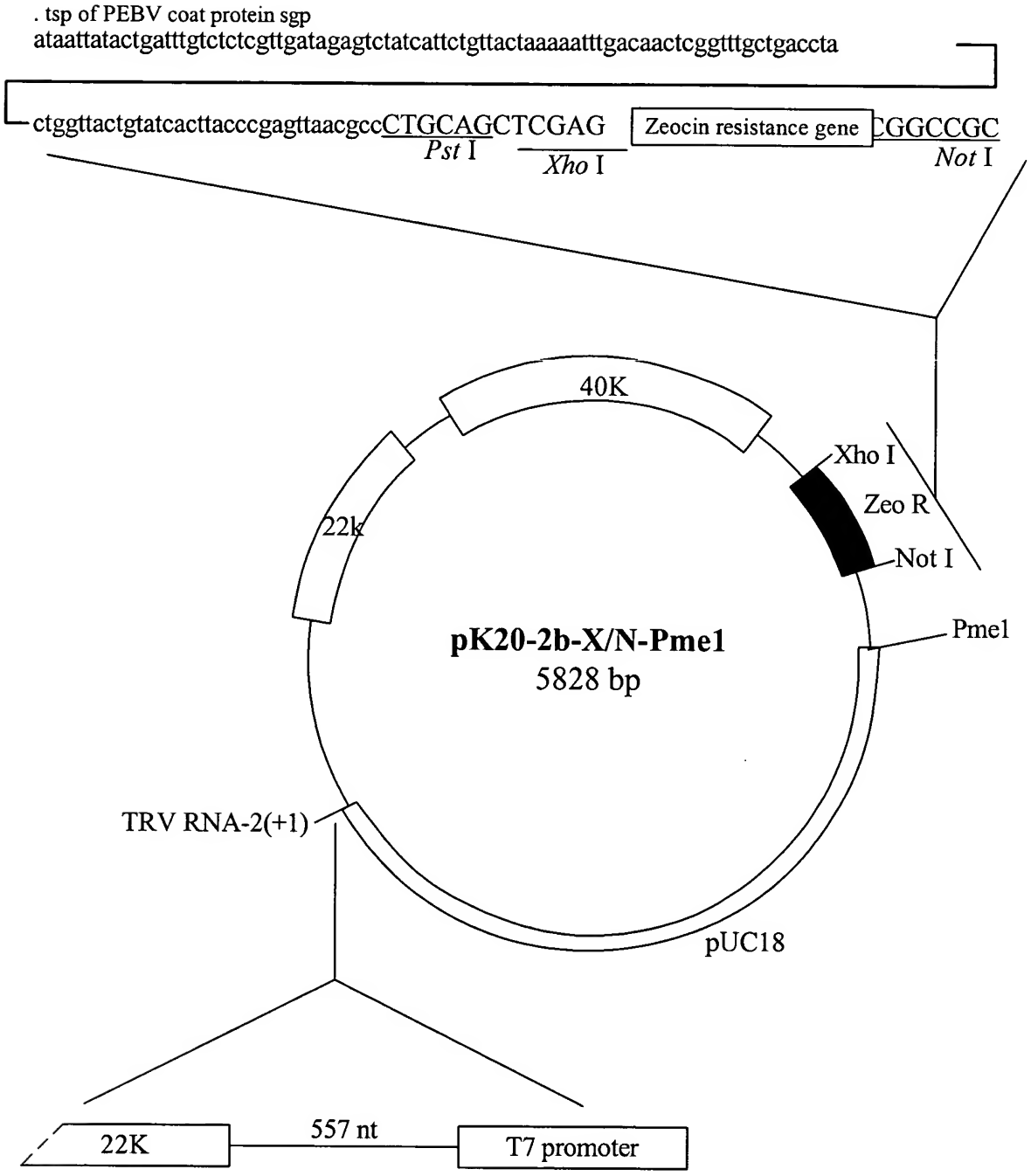
. tsp of PEBV coat protein sgp
ataattatactgatttgtctctcgttgatagagtctatcattctgttactaaaaatttgacaactcggtttgctgaccta
ctggttactgtatcacttacccgagttaacgccCTGCAGCTCGAG
Pst I
Xho I
Zeocin resistance gene
CGGCCGC
Not I
40K
22k
Xho I
Zeo R
Not I
Pme1
pK20-2b-X/N-Pme1
5828 bp
TRV RNA-2(+1)
pUC18
22K
557 nt
T7 promoter

FIG. 5

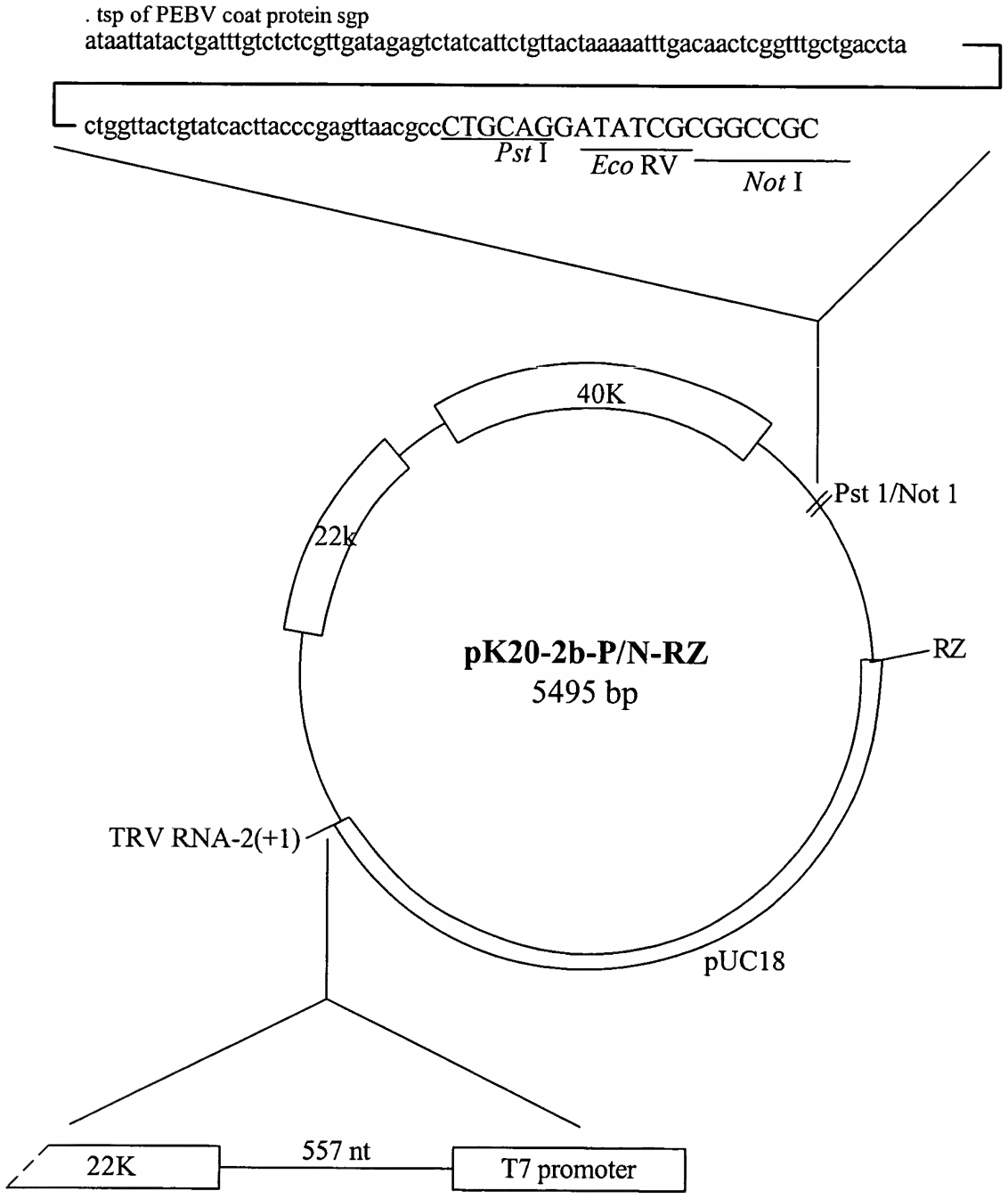
. tsp of PEBV coat protein sgp
ataattatactgatttgtctctcgttgatagagtctatcattctgttactaaaaatttgacaactcggtttgctgaccta
ctggttactgtatcacttacccgagttaacgccCTGCAGGATATCGCGGCCGC
Pst I
Eco RV
Not I
40K
22k
Pst 1/Not 1
pK20-2b-P/N-RZ
5495 bp
RZ
TRV RNA-2(+1)
pUC18
22K
557 nt
T7 promoter

FIG. 6

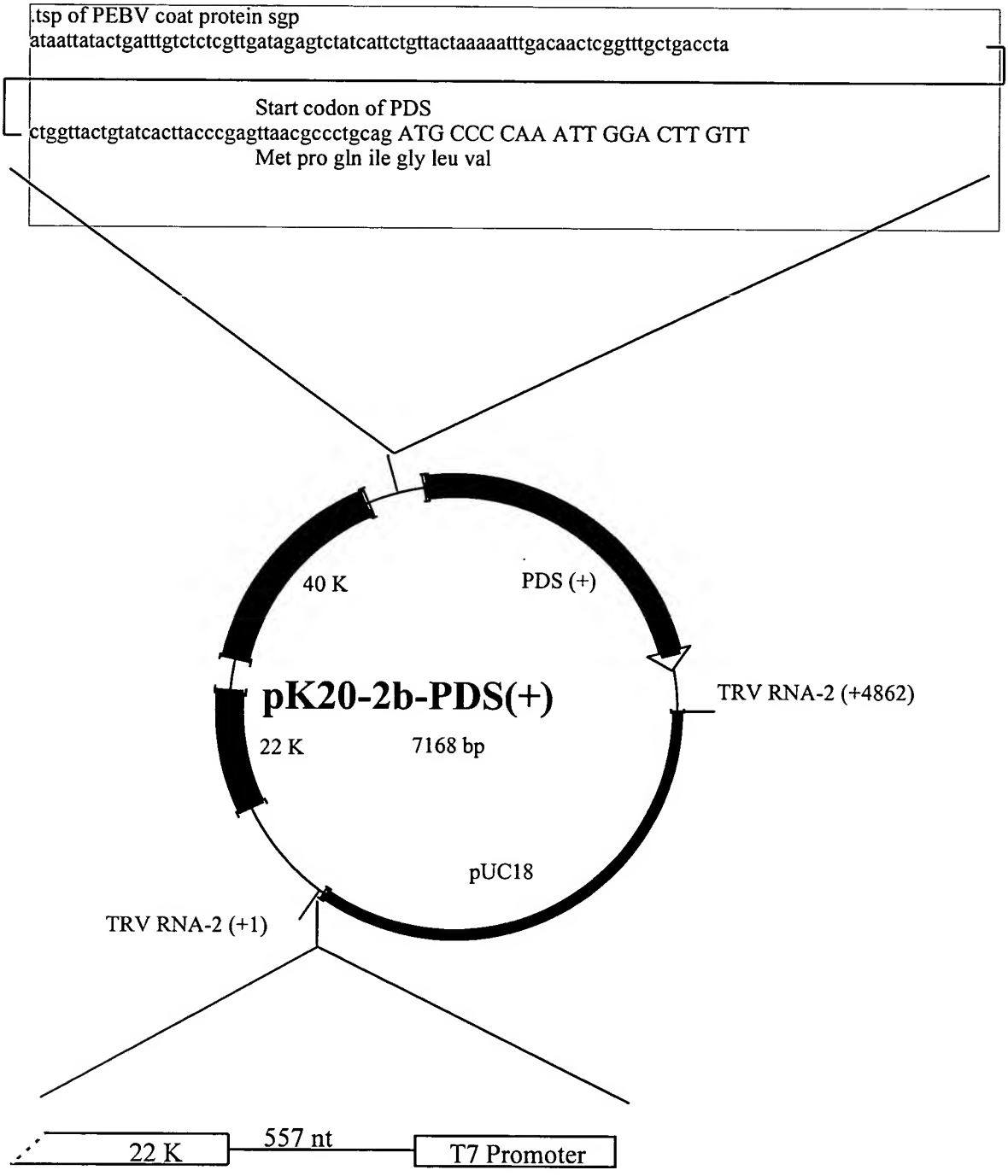
.tsp of PEBV coat protein sgp
ataattatactgatttgtctctcgttgatagagtctatcattctgttactaaaaatttgacaactcggtttgctgaccta
Start codon of PDS
ctggttactgtatcacttacccgagttaacgccctgcag ATG CCC CAA ATT GGA CTT GTT
Met pro gln ile gly leu val
40 K
PDS (+)
pK20-2b-PDS(+)
22 K
7168 bp
TRV RNA-2 (+4862)
pUC18
TRV RNA-2 (+1)
22 K
557 nt
T7 Promoter

FIG. 7

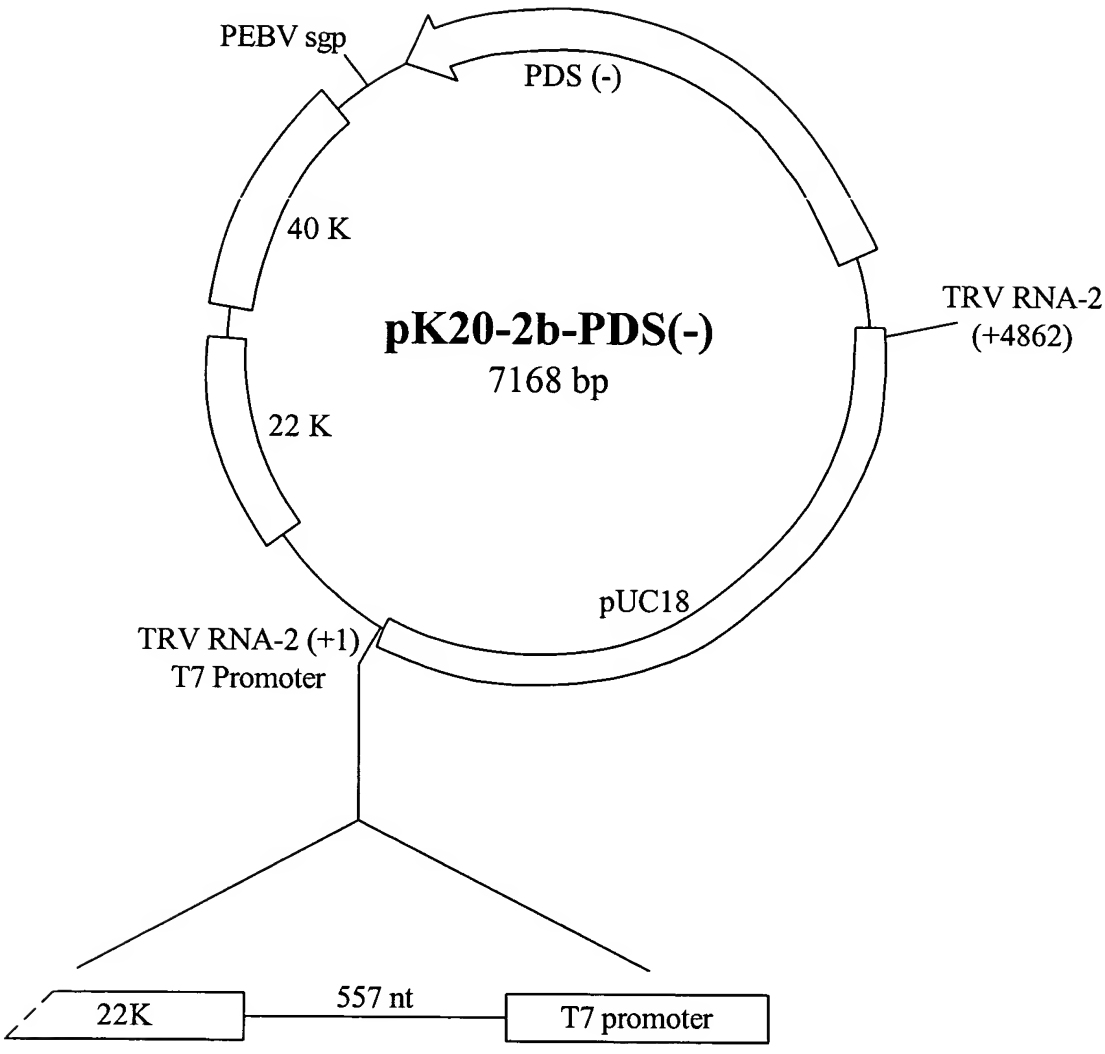
PEBV sgp
PDS (-)
40 K
pK20-2b-PDS(-)
7168 bp
TRV RNA-2
(+4862)
22 K
pUC18
TRV RNA-2 (+1)
T7 Promoter
22K
557 nt
T7 promoter

FIG. 8

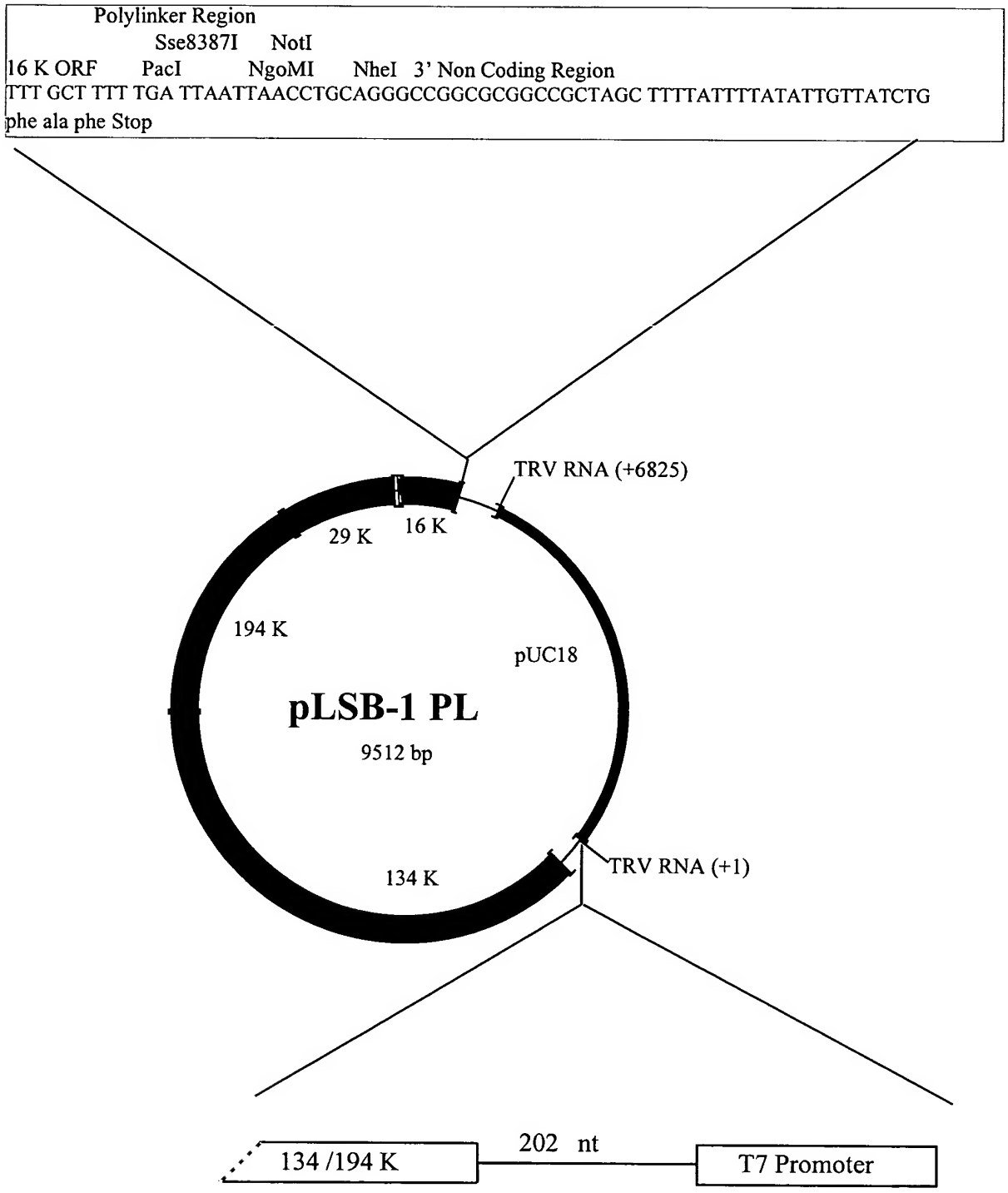
Polylinker Region
Sse8387I NotI
16 K ORF PacI NgoMI NheI 3' Non Coding Region
TTT GCT TTT TGA TTAATTAACCTGCAGGGCCGGCGCGGCCGCTAGC TTTTATTTTATATTGTTATCTG
phe ala phe Stop
TRV RNA (+6825)
29 K
16 K
194 K
pUC18
pLSB-1 PL
9512 bp
134 K
TRV RNA (+1)
134 /194 K
202 nt
T7 Promoter

FIG. 9

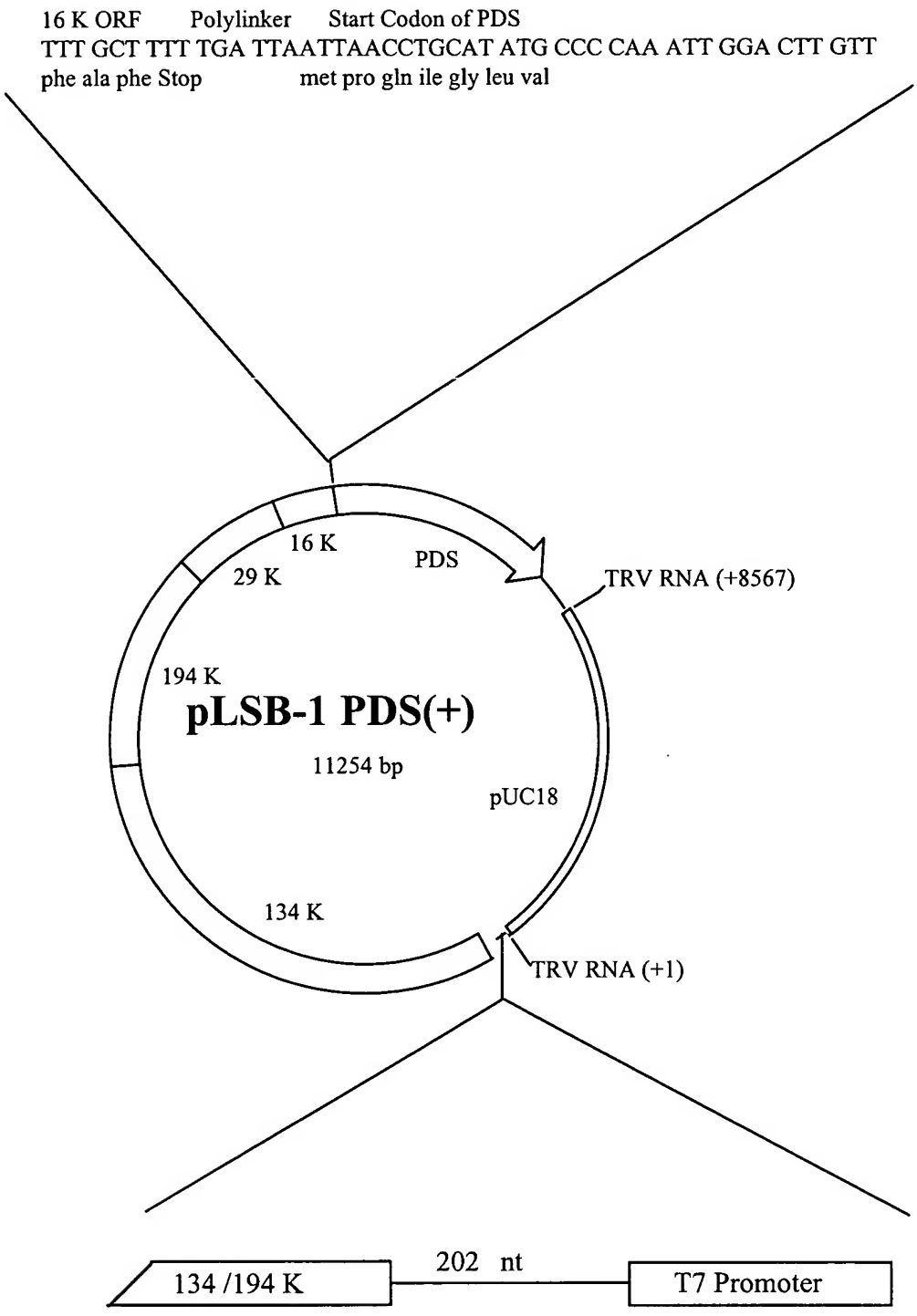
16 K ORF
Polylinker
Start Codon of PDS
TTT GCT TTT TGA TTAATTAACCTGCAT ATG CCC CAA ATT GGA CTT GTT
phe ala phe Stop
met pro gln ile gly leu val
16 K
29 K
PDS
TRV RNA (+8567)
194 K
pLSB-1 PDS(+)
11254 bp
pUC18
134 K
TRV RNA (+1)
134 /194 K
202 nt
T7 Promoter

FIG. 10

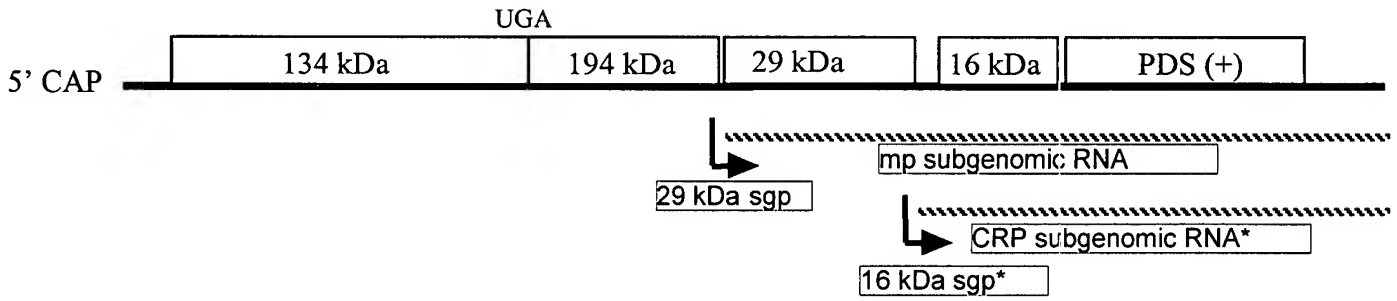
UGA
5' CAP
134 kDa
194 kDa
29 kDa
16 kDa
PDS (+)
mp subgenomic RNA
29 kDa sgp
CRP subgenomic RNA*
16 kDa sgp*

FIG. 11

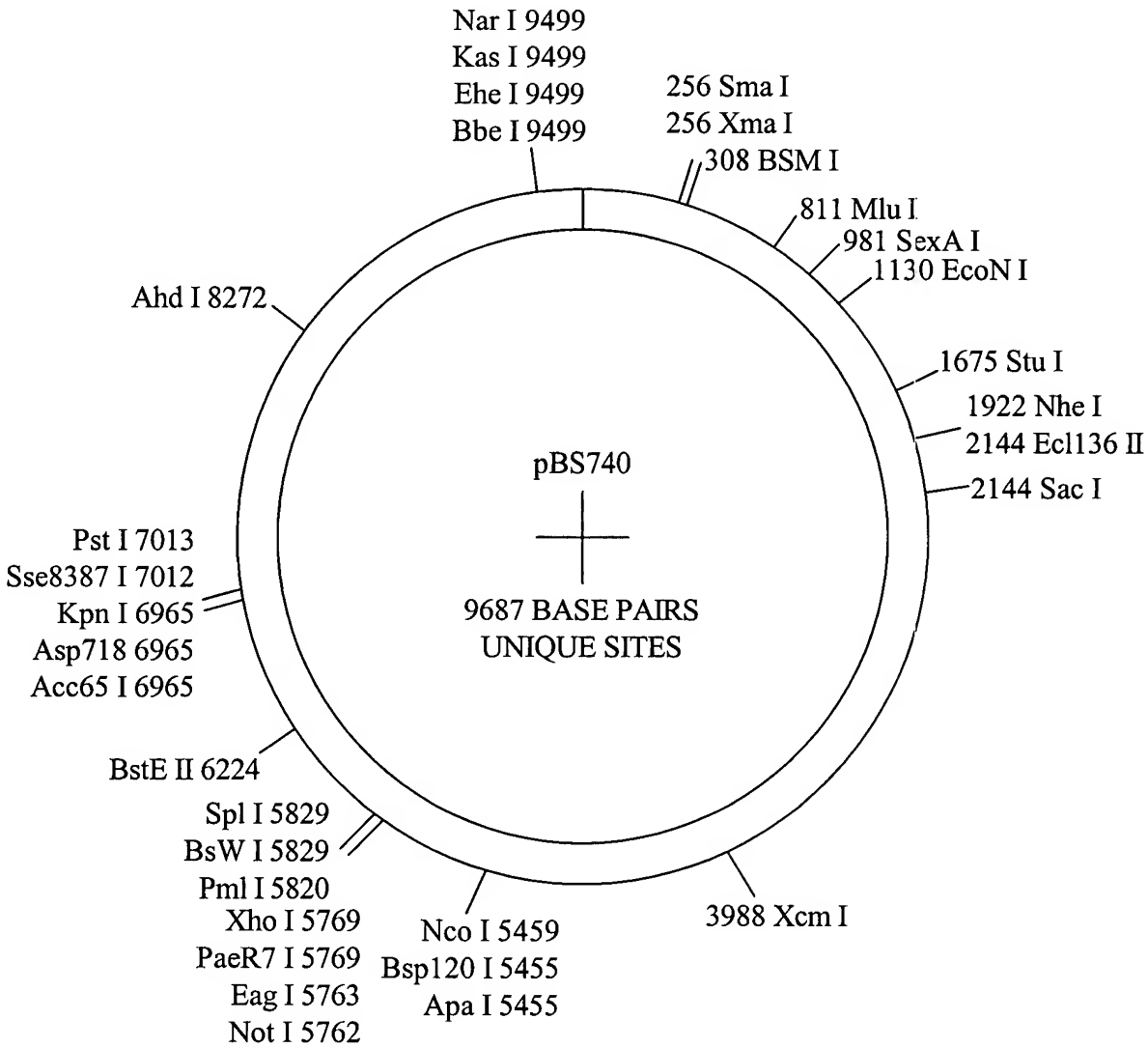
Nar I 9499
Kas I 9499
Ehe I 9499
Bbe I 9499
256 Sma I
256 Xma I
308 BSM I
811 Mlu I
981 SexA I
1130 EcoN I
1675 Stu I
1922 Nhe I
2144 Ecl136 II
2144 Sac I
3988 Xcm I
Nco I 5459
Bsp120 I 5455
Apa I 5455
Spl I 5829
BsW I 5829
Pml I 5820
Xho I 5769
PaeR7 I 5769
Eag I 5763
Not I 5762
BstE II 6224
Pst I 7013
Sse8387 I 7012
Kpn I 6965
Asp718 6965
Acc65 I 6965
Ahd I 8272
pBS740
9687 BASE PAIRS
UNIQUE SITES

FIG. 12

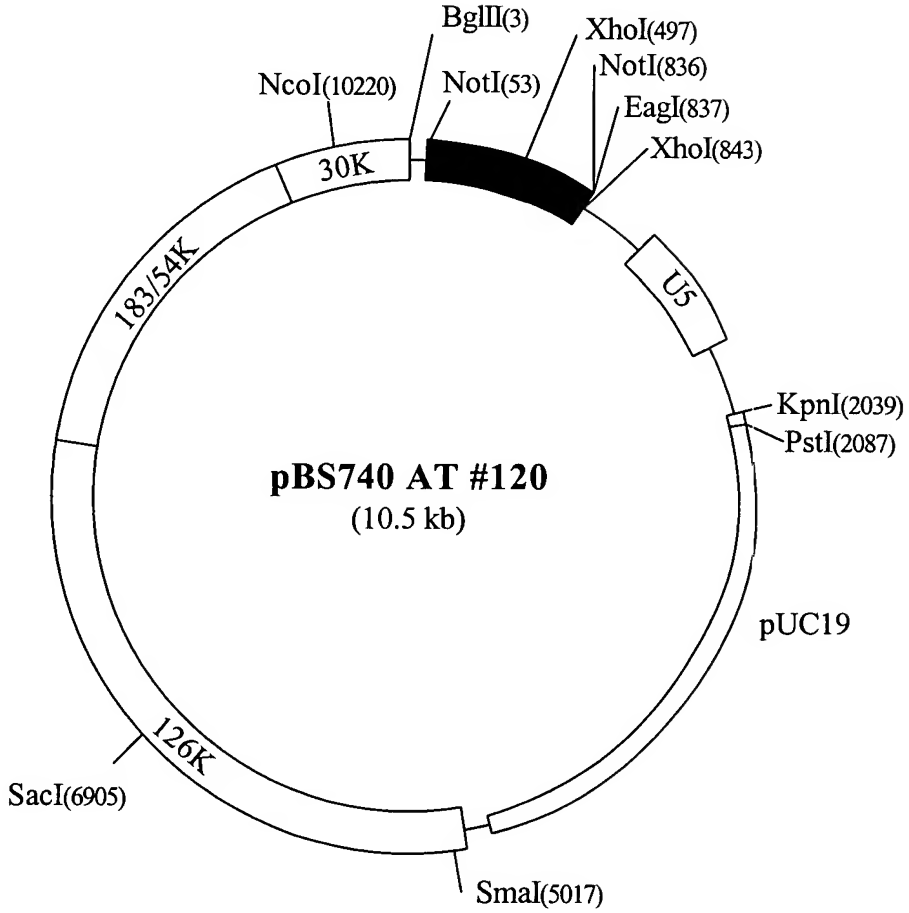
BglII(3)
XhoI(497)
NotI(836)
NcoI(10220)
NotI(53)
EagI(837)
XhoI(843)
30K
183/54K
U5
KpnI(2039)
PstI(2087)
pBS740 AT #120
(10.5 kb)
pUC19
126K
SacI(6905)
SmaI(5017)

FIG. 13

```
740 AT #120 27 TCCGAAACATTCTTCGTAGTGAAGCAAAATGGGGTTGAGTTTCGCCAAGCTGTTTAGCAG
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA042085       TCCGAAACATTCTTCGTAGTGAAGCAAAATGGGGTTGAGTTTCGCCAAGCTGTTTAGCAG

740 AT #120 27 GCTTTTTGCCAAGAAGGAGATGCGAATTCTGATGGTTGGTCTTGATGCTGCTGGTAAGAC
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA042085       GCTTTTTGCCAAGAAGGAGATGCGAATTCTGATGGTTGGTCTTGATGCTGCTGGTAAGAC

740 AT #120 27 CACAATCTTGTACAAGCTCAAGCTCGGAGAGATTGTCACCACCATCCCTACTATTGGTTT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA042085       CACAATCTTGTACAAGCTCAAGCTCGGAGAGATTGTCACCACCATCCCTACTATTGGTTT

740 AT #120 27 CAATGTGGAAACTGTGGAATACAAGAACATTAGTTTCACCGTGTGGGATGTCGGGGGTCA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA042085       CAATGTGGAAACTGTGGAATACAAGAACATTAGTTTCACCGTGTGGGATGTCGGGGGTCA

740 AT #120 27 GGACAAGATCCGTCCCTTGTGGAGACACTACTTCCAGAACACTCAAGGTCTAATCTTTGT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA042085       GGACAAGATCCGTCCCTTGTGGAGACACTACTTCCAGAACACTCAAGGTCTAATCTTTGT

740 AT #120 27 TGTTGATAGCAATGACAGAGACAGAGTTGTTGAGGCTCGAGATGAACTCCACAGGATGCT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA042085       TGTTGATAGCAATGACAGAGACAGAGTTGTTGAGGCTCGAGATGAACTCCACAGGATGCT

740 AT #120 27 GAATGAGGACGAGCTGCGTGATGCTGTGTTGCTTGTGTTT
               | ||||| ||||||||||||||||||||||||||||||
AA042085       GNATGAGNACGAGCTGCGTGATGCTGTGTTGCTTGTGTTT
```

FIG. 14

Nucleotide sequence alignment of 740 AT #120 to *Oryza sativa* D17760

```
740 AT 120   27 AAATGGGGTTGAGTTTCGCCAAGCTGTTTAGCAGGCTTTTTGCCAAGAAGGAGATGCGAA 86
                | ||||||  | |  ||| | ||||||||  ||| |  ||  ||  ||||||||||||||  | |
D17760      166 AGATGGGGCTCACGTTCACGAAGCTGTTCAGCCGCCTCTTCGCCAAGAAGGAGATGAGGA 225

740 AT 120 87  TTCTGATGGTTGGTCTTGATGCTGCTGGTAAGACCACAATCTTGTACAAGCTCAAGCTCG 146
                | ||  |||||  |||||  |||||  ||  |||||  |||||  |||  |  ||||||||||||||
D17760      226 TCCTCATGGTCGGTCTCGATGCGGCCGGTAAAACCACCATCCTCTACAAGCTCAAGCTCG 285

740AT 120   147 GAGAGATTGTCACCACCATCCCTACTATTGGTTTCAATGTGGAAACTGTGGAATACAAGA 206
                | |||||  ||||||||  |||||  ||  ||  |||||  |||||  ||||||||  ||  |||||||
D17760      286 GCGAGATCGTCACCACTATCCCCACCATCGGTTTTAATGTCGAAACTGTTGAGTACAAGA 345

740 AT 120 207 ACATTAGTTTCACCGTGTGGGATGTCGGGGGTCAGGACAAGATCCGTCCCTTGTGGAGAC 266
                |||||||  ||||||||  ||||||||  ||  ||||||||||||||  |  |||  |||||||  |
D17760      346 ACATTAGCTTCACCGTTTGGGATGTTGGTGGTCAGGACAAGATCAGGCCCCTGTGGAGGC 405

740 AT 120 267 ACTACTTCCAGAACACTCAAGGTCTAATCTTTGTTGTTGATAGCAATGACAGAGACAGAG 326
                ||||  ||||||||||  ||  ||  ||  ||||||||  ||  ||||||||||||||  | |
D17760      406 ACTATTTCCAGAACACCCAGGGCCTCATTTTTGTTGTGGACAGCAATGACAGAGAGCGTG 465

740 AT 120 327 TTGTTGAGGCTCGAGATGAACTCCACAGGATGCTGAATGAGGACGAGCTGCGTGATGCTG 386
                ||||||||||  |  |||||  ||||||  |  ||||||||||||||  |||||  ||||||||||
D17760      466 TTGTTGAGGCCAGGGATGAGCTCCACCGTATGCTGAATGAGGATGAGCTACGTGATGCTG 525
```

FIG. 15A

```
740 AT 120 387 TGTTGCTTGTGTTTGCCAACAAGCAAGATCTTCCAAATGCTATGAACGCTGCTGAAATCA 446
               || |||| |||||||| ||||| |||||||||||| ||||| |||||||||||||| ||||
D17760     526 TGCTGCTGGTGTTTGCAAACAAACAAGATCTTCCTAATGCCATGAACGCTGCTGAGATCA 585

740 AT 120 447 CAGATAAGCTTGGCCTTCACTCCCTCCGTCAGCGTCATTGGTATATCCAGAGCACATGTG 506
               | || |||||||| || |||||| | || ||||| || ||||| ||||||||||||||||
D17760     586 CCGACAAGCTTGGTCTGCACTCCTTGCGCCAGCGGCACTGGTACATCCAGAGCACATGTG 645

740 AT 120 507 CCACTTCAGGTGAAGGGCTTTATGAAGGTCTGGACTGGCTCTCCAACAACATCGCTGGCA 566
               | || || ||||| ||| | ||||| || || |||||||| |||||||||| ||   ||
D17760     646 CTACCTCTGGTGAGGGGTTGTATGAGGGGCTTGACTGGCTTTCCAACAACATTGCCAACA 705

740 AT 120 567 AGGCATGATG 576
               |||| ||| |
D17760     706 AGGCTTGAAG 715
```

FIG. 15B

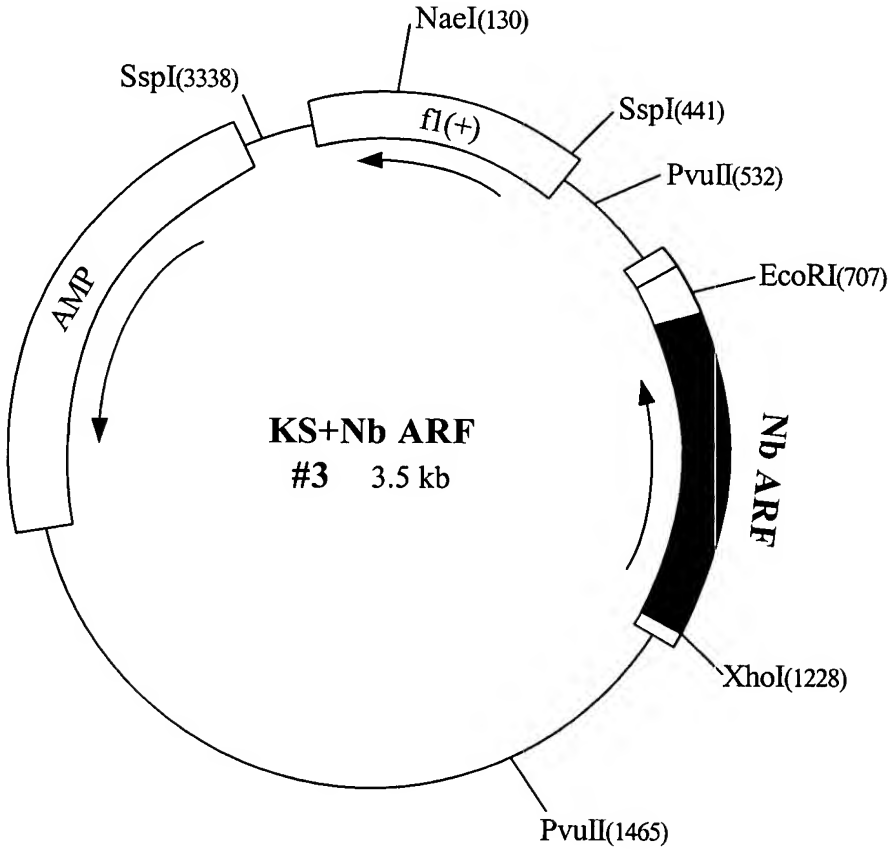
NaeI(130)
SspI(3338)
SspI(441)
f1(+)
PvuII(532)
EcoRI(707)
AMP
KS+Nb ARF
#3 3.5 kb
Nb ARF
XhoI(1228)
PvuII(1465)

FIG. 16

```
740 AT #120  TGGTCTTGATGCTGCTGGTAAGACCACAATCTTGTACAAGCTCAAGCTCGGAGAGATTGT
             ||||||||||| |||||||| ||||| || |||||||||||||||||| |||||||| ||
Nb  ARF #3   CGGTCTTGATGCAGCTGGTAAAACCACCATATTGTACAAGCTCAAGCTGGGAGAGATAGT

740 AT #120  CACCACCATCCCTACTATTGGTTTCAATGTGGAAACTGTGGAATACAAGAACATTAGTTT
             |||||  || |||||  |||||  |||||||||||  |||||  |||||||||||||  ||  ||
Nb  ARF #3   TACCACTATTCCTACCATTGGATTCAATGTGGAGACTGTTGAATACAAGAACATAAGCTT

740 AT #120  CACCGTGTGGGATGTCGGGGGTCAGGACAAGATCCGTCCCTTGTGGAGACACTACTTCCA
             |||  ||  ||||||||  ||  ||||||||||||||||||  ||  ||||||||  ||  ||||||||
Nb  ARF #3   CACGGTCTGGGATGTTGGTGGTCAGGACAAGATCCGACCATTGTGGAGGCATTACTTCCA

740 AT #120  GAACACTCAAGGTCTAATCTTTGTTGTTGATAGCAATGACAGAGACAGAGTTGTTGAGGC
             |||||  |||||  ||  ||||||||  ||  |||||  |||||   |  ||   |  ||||||||||
Nb  ARF #3   AAACACACAAGGACTTATCTTTGTGGTCGATAGTAATGATCGTGATCGTGTTGTTGAGGC

740 AT #120  TCGAGATGAACTCCACAGGATGCTGAATGAGGACGAGCTGCGTGATGCTGTGTTGCTTGT
             |  |||||||  ||  |||  |||||  ||||||||||  ||  |||  |  |||||||||  |||||||
Nb  ARF #3   TAGAGATGAGCTGCACCGGATGTTGAATGAGGATGAACTGAGGGATGCTGTGCTGCTTGT

740 AT #120  GTTTGCCAACAAGCAAGATCTTCCAAATGCTATGAACGCTGCTGAAATCACAGATAAGCT
             ||||||  ||||||||||||||||||||||||||||||  ||||||||  ||  ||  ||  |||||
Nb  ARF #3   GTTTGCTAACAAGCAAGATCTTCCAAATGCTATGAATGCTGCTGAGATTACTGACAAGCT

740 AT #120  TGGCCTTCACTCCCTCCGTCAGCGTCATTGG
             |||  |||||  ||  ||||||||  |||||  |||
Nb  ARF #3   TGGTCTTCATTCTCTCCGTCAACGTCACTGG
```

FIG. 17

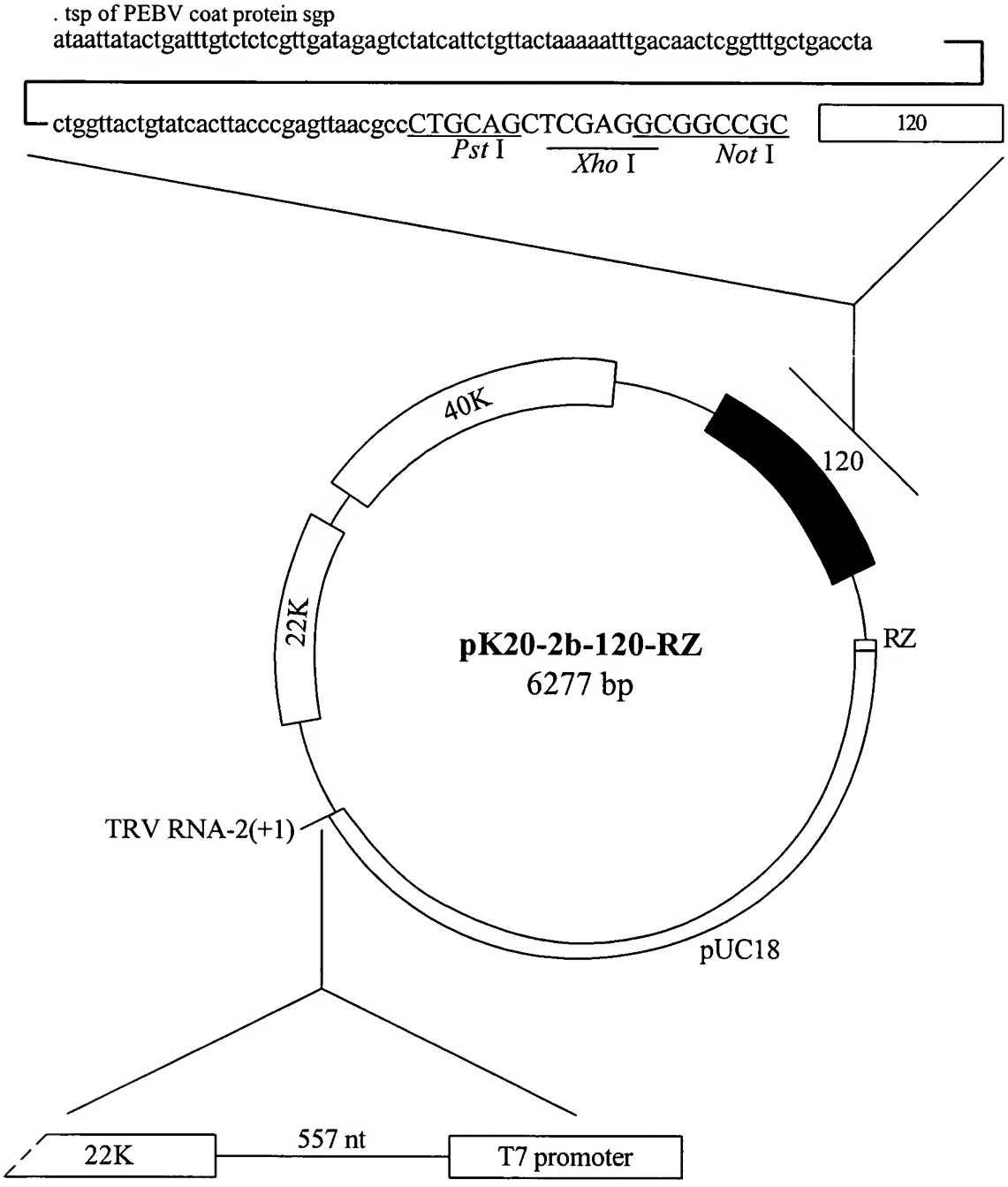
. tsp of PEBV coat protein sgp
ataattatactgatttgtctctcgttgatagagtctatcattctgttactaaaaatttgacaactcggtttgctgaccta
ctggttactgtatcacttacccgagttaacgccCTGCAGCTCGAGGCGGCCGC
Pst I
Xho I
Not I
120
40K
120
22K
pK20-2b-120-RZ
6277 bp
RZ
TRV RNA-2(+1)
pUC18
22K
557 nt
T7 promoter

FIG. 18

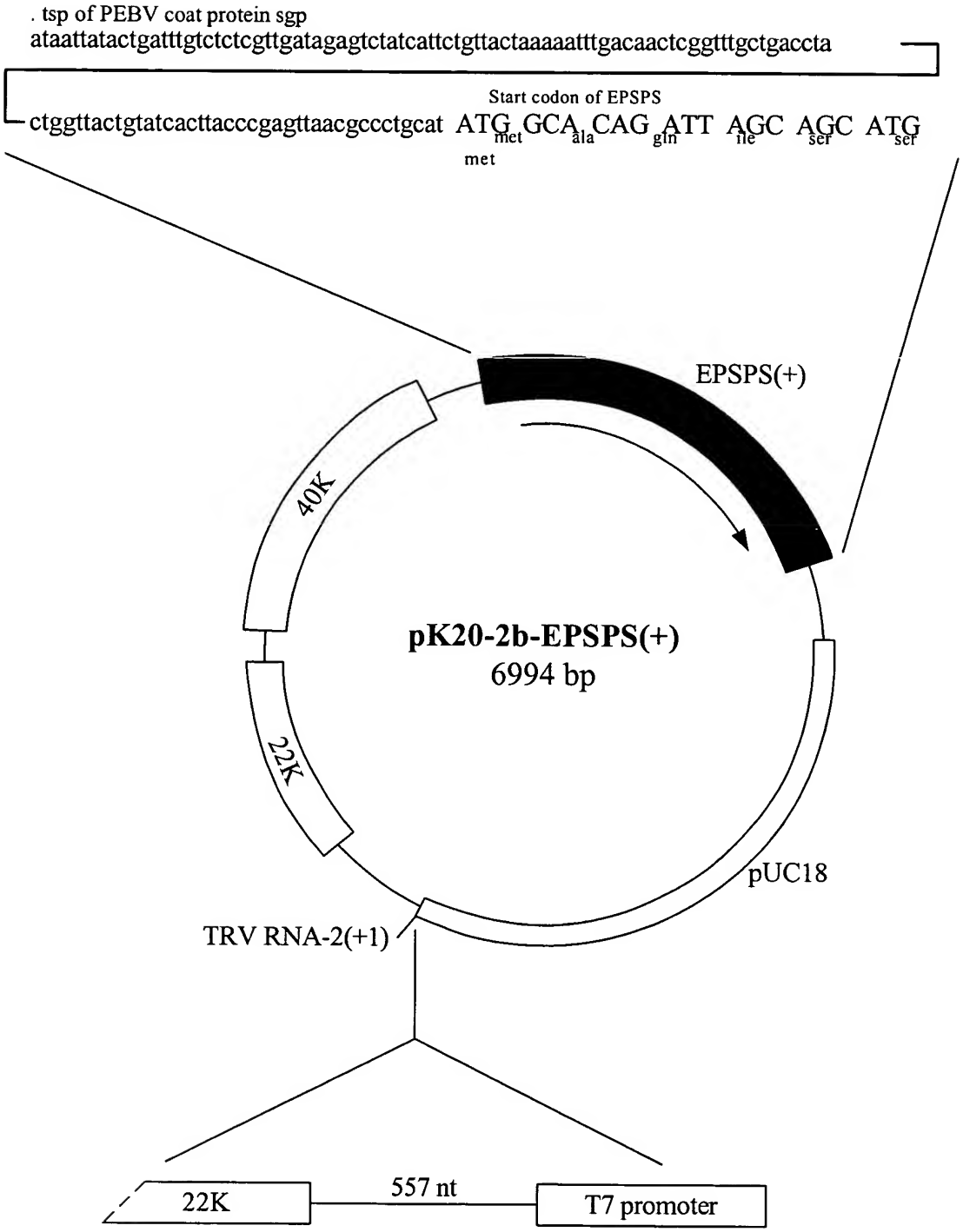
. tsp of PEBV coat protein sgp
ataattatactgatttgtctctcgttgatagagtctatcattctgttactaaaaatttgacaactcggtttgctgaccta
Start codon of EPSPS
ctggttactgtatcacttacccgagttaacgccctgcat ATG GCA CAG ATT AGC AGC ATG
met ala gln ile ser ser
met
EPSPS(+)
40K
pK20-2b-EPSPS(+)
6994 bp
22K
pUC18
TRV RNA-2(+1)
22K
557 nt
T7 promoter

FIG. 19

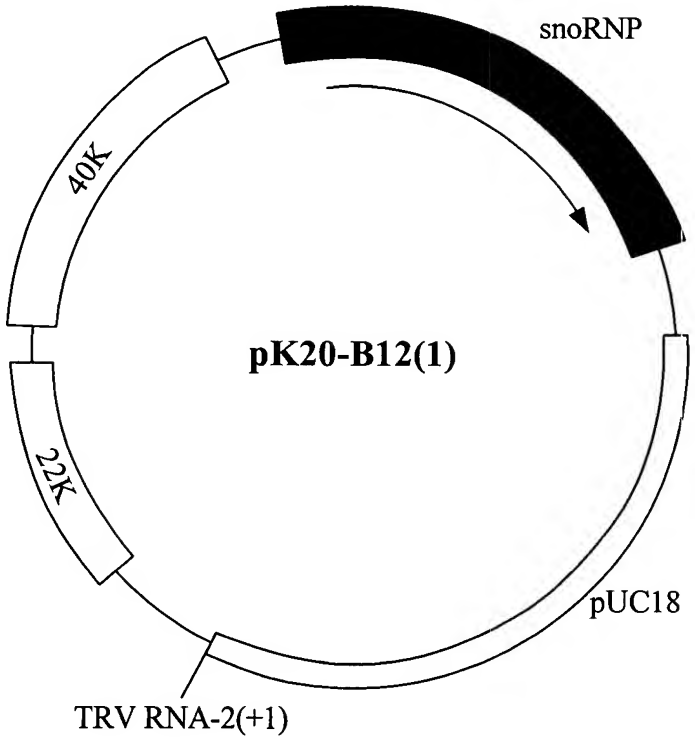

FIG. 20

GAAACCGGCGAAGCAGCTCAGGTCACAATTTAGCGAGGATGTATCTCCAGTTTTA
CATCAATGAGAATGGTGACAAAGTTTACACCACTAAGAAAGAGTCACCACTGGGT
TTGGCCACAGAATCCGCTCACCCAGCCCGCTTTTCCCCCGATGATAAATATTCAA
GGCAAAGAGTGCTTCTGAAGAAGCGATTTGGTTTGCTTCCAACCCAAAAGCCACC
TCAAAAGTACTAAAAGTTTTTGCTATTGTGTATTGCTTTCTACTCATGGTTATTA
TGTTTCTCTGTCTTGTCGTTGTTGACGTGACTCTTGTATTGCAACTCAAATTGC
ATGGCAGCAATTCAAACCTCATATCTAATTG

FIG. 21

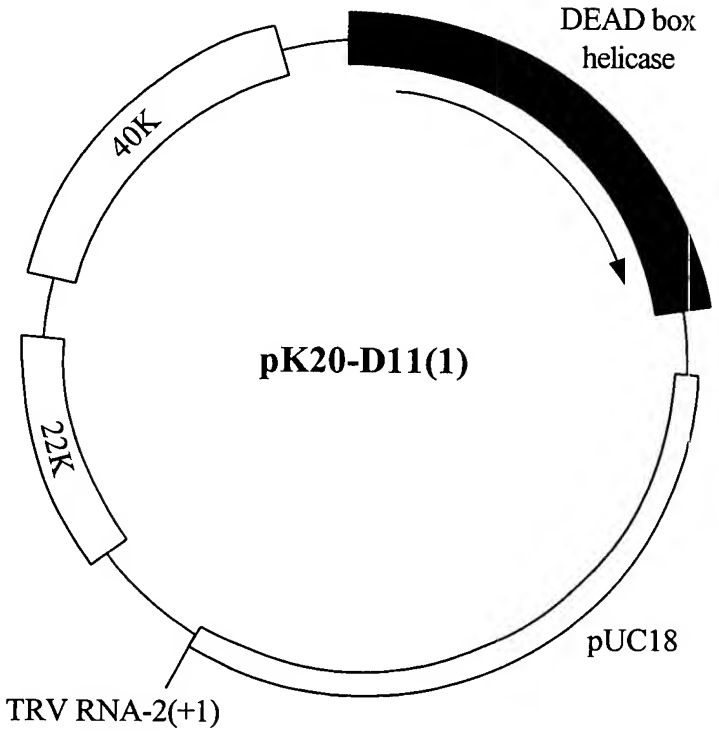

FIG. 22

GCCCACGCGTCCGATGAGGCCAAGTTGACCCTTCATGGACTTGTACAGCACTACA
TTAAATTGAGTGAAACCGAGAAAAACCGGAAACTAAATGATCTGCTGGACGCCTT
AAACTTCAACCAAGTTGTTATATTTGTCAAGAGTGTAAGTCGGGCAGCACAGCTG
GATAAATTACTAGTGGAGTGTAATTTTCCATCTATCTGCATCCACTCTGGCATGA
CGCAGGAAGAAAGATTGACTCGCTACAAGGGTTTCAAGGAGGGCCACAAGAGAAT
TCTTGTCGCAACTGATCTGGTTGGTAGGGGCATTGACATCGAAAGGGTCAACATT
GTTATTAACTATGACATGCCAGATTCTGCAGACACGTATCTTCACAGAGTGGGTC
GAGCTGGTAGGTTTGGAACTAAAGGCCTTGCCATCACATTTGTGTCATCTGCATC
AGATTCTGATGTTCTAAATCAGGTTCAAGAAAGGTTTGAAGTAGACATAAAAGAG
CTTCCTGAGCAGATTGATACTTCTACGTACATGCCATCTTAGCGATCTCGAGAGC
TTCCAGCAATATCAAGTCATTTAAAAGATGGGGGGAACTGACAGGTGTTTTGCTA
TTGTTGTTAATTTGAAGAATTGGGGGGCTCCTACTATATGCTCTTGCACTGCTGA
GCTGCTGTACCCTTGTTGAACTACTCTTTCTCCTCCAGTTTAAGAGGAGCACCTA
ACAAATG

FIG. 23

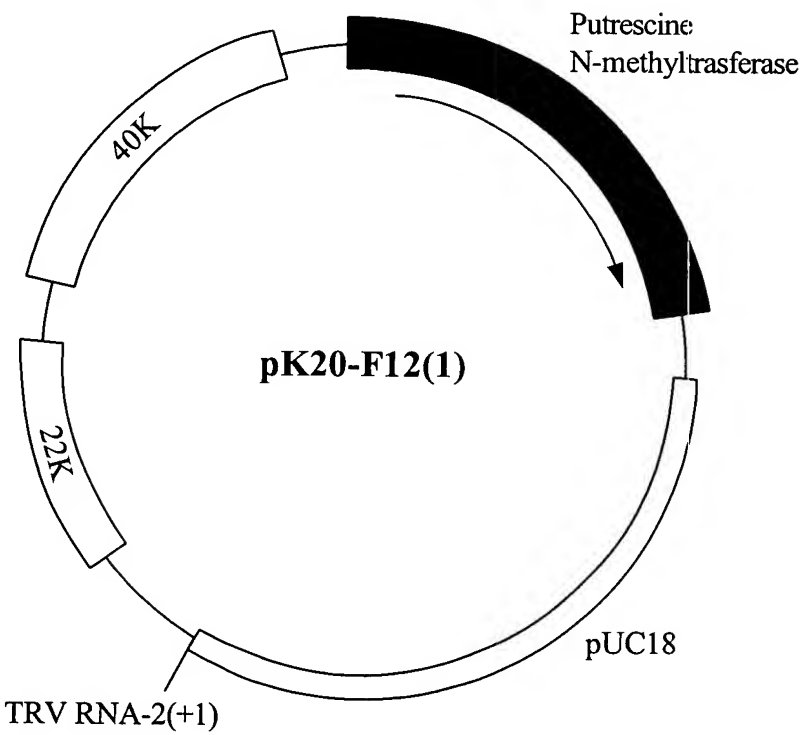
Putrescine
N-methyltrasferase
40K
pK20-F12(1)
22K
pUC18
TRV RNA-2(+1)

FIG. 24

GGTCAAATCCAAATTAGCACCTCTCAAGTTCTACAACTCTGATATTCACAAAGCA
CCATTCATTTTGCCATCTTTCGCCAGAAGTATGATCGAGTCTTAATCAAGTGAAC
AATGAACACTGGTGGTACAATCATTGGACCAAGATCGAGTCTTTATCAAGTGAAT
AAATAAAGTGAAATGCAACGCATTGTATGAATCCAGTAGTAATTATCATAATTCG
GATTCACCAATTAGTGTAAATTCTTTCTGTGGTGTTTGGTTTTTTCATATAAATT
TTCTTGCTGTTGTTTTGATATGACGTTTCAACTCAATCCACGCAAATCATTTCAT
T

FIG. 25

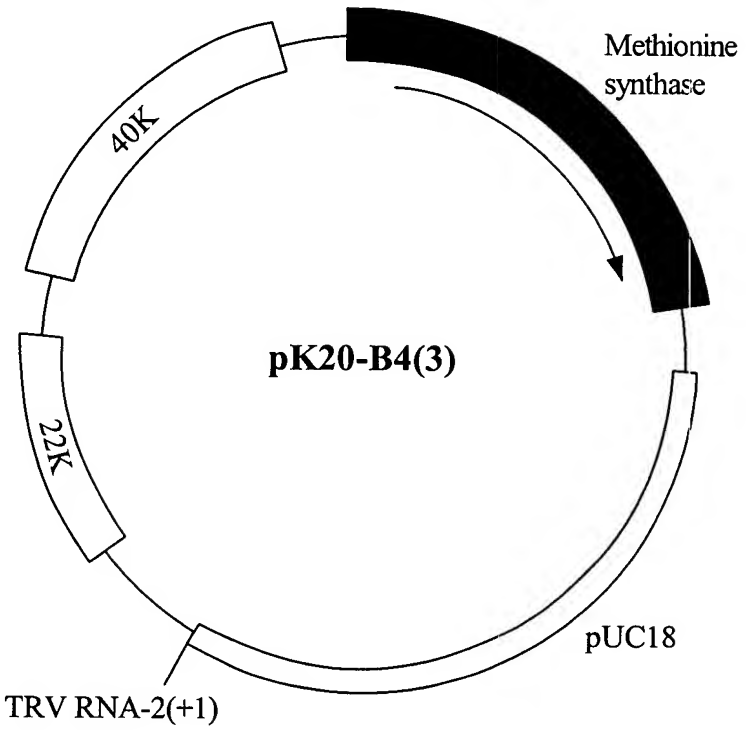

FIG. 26

GGCCTTTTACTTGAACTGGGCTGTCCACTCCTTCAGAATCACCAACGTCGGCATT
CAAGACACCACCCAGATCCACACACACATGTGCTACTCCAACTTCAATGACATTA
TCCACTCTATCATTGACATGGATGCTGATGTGATCACAATTGAGAACTCACGGTC
CGATGAGAAGCTCCTCTCAGTTTTCAGGGAGGGAGTTAAGTATGGTGCTGGAATT
GGCCCCGGTGTCTATGATATCCACTCCCCTAGAATACCATCAACGGAAGAGATTG
CTGACAGAGTTAACAAGATGCTTGCTGTTCTTGACACCAACATCTTGTGGGTCAA
CCCAGATTGTGGTCTCAAGACTCGCAAGTACGCTGAGGTAAAGCCAGCCCTCGAG
AACATGGTTTCTGCTGCCAAGGCCATCCGCACCCAACTTGCCAGCACCAAGTGAG
TCAGATGAAGGAGTCGCGACATATCAAGATTCCCTTTTTCATGAAACAGAAAATT
CTATGTTGATTTTTAATCATTGTGTTGGCAACAAATATTGTTGTGTAGGTTAGCT
CTGCCCGCTGGGCATTTTCTTCTTGTGTTTGAGCCATTTCCTTTTCGGAAGAAAA
TATATCCAATGTATTATGATGTTTTATGGGTCGATTTTGGTTAC

FIG. 27

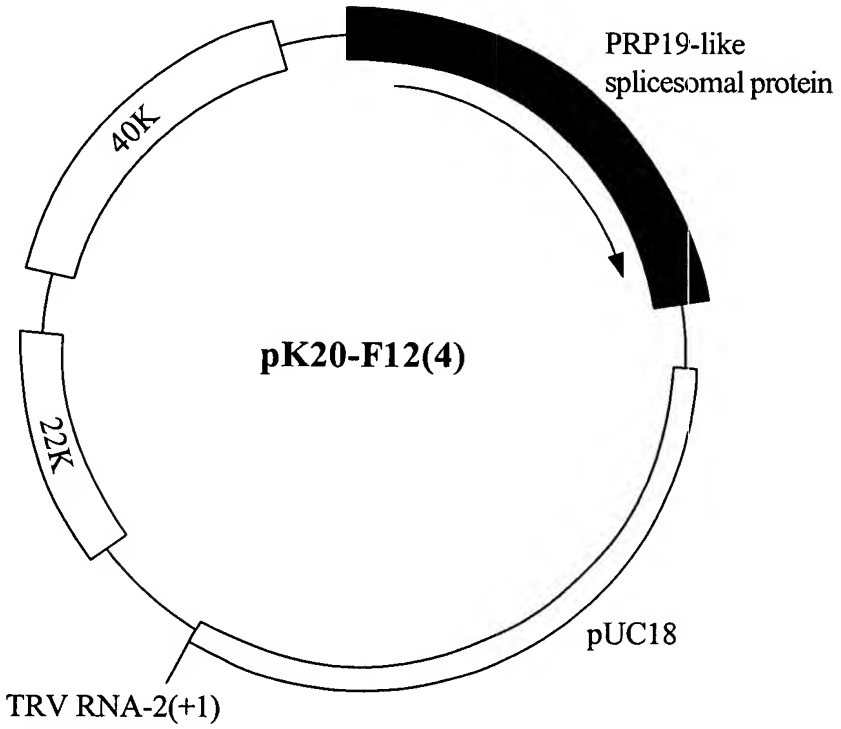
PRP19-like
splicesomal protein
40K
pK20-F12(4)
22K
pUC18
TRV RNA-2(+1)

FIG. 28

GGATGTGTTGATCAATGGGAAAAGAGCTGCTGAGGACGAGGAGATGGGTCCTGAT
GGCAAGAAAATTCGCCCTGGAATATCAAACTCTGTCATTGAGACTCTTACGGAAT
GTAATGCTGCTCTTTCACAGCAAAGGAAAAGACGACAGATACCGGCAACACTGGC
CTCTGTGGATGCTCTGGAAAGATATACCCAACTGAATAGTTATCCTCTTCACAAA
ACCAACAAACCTGGTATTTTGTCTTTGGATATTCATTATCCTAAGGACTTAATTG
CTACTGGTGGTGTTGATTCAAATGCTGTGGTCTTTGATCGTCCTTCAGGACAAAT
GAGGGTGAACTAGTGGTCTCTGGCTCAGCAGATAAGACAGTTCGTTTGTGGCAAA
GTTCTGAAAATGGGAACTATGACTGTAGGCATGTCTTGAAAGATCATACAGCAGA
GGTGCAAGCTGTCACTGTCCATGCAACCAATAACTATTTTGTGACTGCTTCTCTT
GATAGCACATGGTGCTTTTATGATCTTGCTTCTGGCTTATGCCTTGCACAGGTGG
CAGATGCTACAGAATCTGAGGGTTACACATCCGCAAGCTTTCCCACCCTGATTGG
TCTTGATCCTTGGGAACAGGGACCTCAGGGTCTCTGGTTCAGATTTTGGGATTGT
AAAAAGTCCAGG

FIG. 29

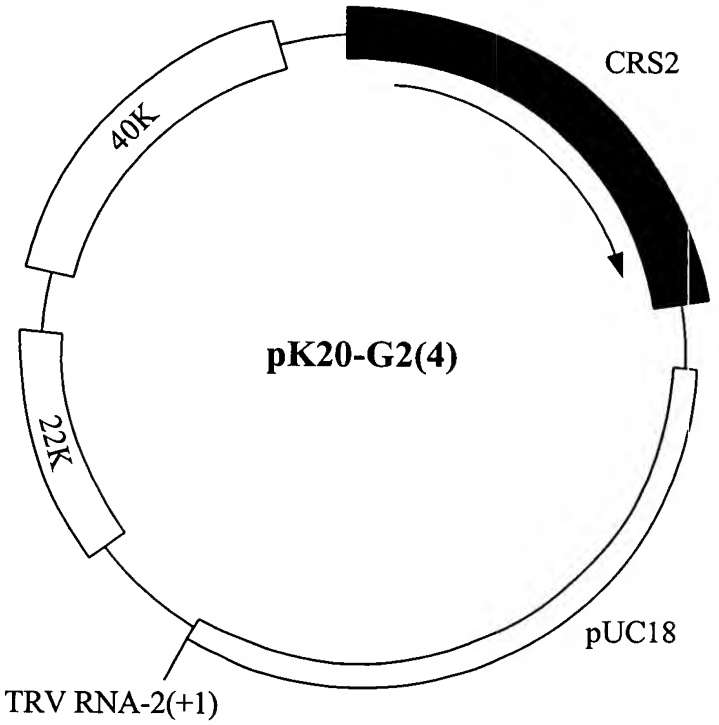

FIG. 30

GCTCCCAGAGCCTAATGGGGTTAAGTTTGAGTACACTCCTTGGTTAATTGTCGGA
TNGGGAAATCCCGGTAACAAGTATCATGGGACTCGCCACAATGTTGGTTTTGAAA
TGATTGATCGAGTTTCTCAAGAGGAGGGAATCGTATTAAACACAATACAGTCAAA
GGCTTTGATAGGAATAGGTTCGATAGGGGAGGTACCTGTGGTATTGGCAAAGCCT
CAAGCCTACATGAATTTCAGTGGAGAATCGGTCGGACCACTTGCTGCATATTATC
AGGTGCCTCTGCGTCACATCCTTCTGGTTTATGATGAGATGAGCTTACCAAATGG
TGTTCTGAGGCTTCAGCCTAAAGGAGGACATGGCCAGCATAATGGGGTGAAAAGT
GTGATGGAGCATTTGGATTGTCGCAGGGAATTTCCCCGATTTTGCATAGGCATAG
GAAATCCACCTGGAACTATGGACATGAAGGCATATCTTCTACAGAAATTCAGTGA
TACAGAGCGGAAGCAGGTGGATGCAGCACTTAATCAAGGAGTTGATGCTGTCAGG
ACGGTAGTATTGGAAGGCTTTGGTAGTAAAATTTCACGATTTAATATAGGACAGA
AATACAAGTATCACAAAGTTTGATGAAATTGAATCTAAAATGAAGGTGTAAAAGA
GCACGAAGATTTACTGATAACTTCAAGTCTAAAAATTAAGGGTGTAAAAAGACCC
CAAGG

FIG. 31